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Applicant: Bruno Tocque et al. Customer No: 21559

Serial No:

Filed: March 6, 2002

Titled: GENETIC MARKERS OF TOXICITY, PREPARATION AND USES

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PRELIMINARY AMENDMENT

Prior to examination, kindly amend the above-referenced application as follows:

In the Specification

On page 1, after the title, insert the following paragraph:

--Cross Reference To Related Applications

This application is a continuation-in-part of U.S.S.N. 09/456,370, filed December 8, 1999. This application also claims priority from French application

42. The method according to claim 40, wherein the nucleic probes a) are cDNA or cDNA fragments prepared from RNAs of treated and untreated cells.

43. The method according to claim 40, wherein the nucleic probes a) are amplification products.

44. The method according to claim 40, wherein the nucleic probes a) are labeled by radioactive, fluorescent, enzymatic or colorimetric labels.

45. The method according to claim 40, wherein the test compound is an individual compound or is present in a mixture with other substances.

46. The method according to claim 40, wherein the library b) further comprises nucleic acid clones specific for genes whose level of expression is modified in a situation of apoptosis.

47. The method according to claim 40, wherein the library b) is prepared by (i) hybridizing a first nucleic acid population from a mammalian cell in a situation of apoptosis and a second nucleic acid population from a cell in a control situation and (ii) separating, from the hybrids formed, nucleic acids comprising an unpaired region.

serial no. 99/11405, filed September 13, 1999, and international application serial no. PCT/FR00/02503, filed on September 12, 2000.--

In the claims

Please cancel claims 1-39 and add the following new claims:

40. A method of analysis of the toxic potential of a test compound, said method comprising separately contacting, under conditions allowing hybridisation to occur,

a) labeled nucleic acid probes corresponding to RNA molecules from mammalian cells treated with said test compound on the one hand and from untreated mammalian cells on the other hand, with

b) a library of nucleic acids, wherein said library comprises, immobilized on a support, nucleic acid clones specific for splicing forms of genes, said splicing forms being characteristic of apoptosis,

the hybridization profile indicating the toxic potential of the test compound.

41. The method according to claim 40, wherein the nucleic probes a) correspond to messenger RNAs from treated and untreated cells.

48. The method according to claim 47, wherein the situation of apoptosis is produced by induction or enhancement, in said mammalian cell, of the activation, preferably of the expression of an anti-oncogene.

49. The method according to claim 48, wherein the anti-oncogene is selected from p53, Rb, p73, myc, TUPRO-2 and NHTS.

50. The method according to claim 40, wherein the library b) comprises nucleic acid clones specific for at least a part of a gene selected from the following genes: Aldolase A; S4 subunit of proteasome 26S; Alpha-tubulin; Glucosidase II; lamin B receptor homologue; EF1-alpha; Fra-1; tyrosine kinase AX1 receptor; spliceosomale Protein SAP62; TRAF-3; EF2; TEF-5; CDC25b; interleukine-1 receptor-associated kinase (« IRAK »); WAF-1; c-fos (exon 4); ckshs1; PL16; NFAR-2; phosphatidylinositol4-kinase, ERF, Eph type receptor tyrosine kinase (hEphB1b); BAF60b protein of the SWI/SNF complex; EB1; MSS1; retinoïc acid alpha receptor (RARa); translation initiation factor eiF4A; STE20 type kinase; protein HSP 90kda; Lipocortin II; protein TPT1 (« translationally controlled tumor proteon »); Hsc70; Cytokeratin 18; 2-oxoglutarate dehydrogenase; mitochondrial gene NADH6; mitochondrial gene NADH deshydrogenase 4; alpha subunit of mitochondrial ATP synthase.

51. The method according to claim 40, wherein the library b) comprises at least 1 clone of sequence selected from SEQ ID Nos: 1 to 37.

52. The method according to claim 51, wherein the library b) comprises at least 5 clones of sequence selected from SEQ ID Nos: 1 to 37.

53. The method according to claim 40, wherein the treated or untreated cells are of human origin.

54. The method according to claim 40, wherein the treated or untreated cells are cell lines.

55. The method according to claim 40, wherein the treated or untreated cells are primary cultures.

56. A method of diagnosis of the toxic potential of a test compound, said method comprising contacting, under conditions allowing hybridisation to occur:

(i) labelled nucleic acid probes corresponding to mRNA molecules from untreated mammalian cells and a library of immobilized nucleic acids, wherein the library comprises different nucleic acid clones comprising a sequence complementary to at least a portion of a gene that is spliced or whose expression is altered during apoptosis in a mammalian cell, and

(ii) labelled nucleic probes corresponding to mRNA molecules from mammalian cells treated with said test compound and said nucleic acid library, the hybridization profile indicating the toxic potential of the test compound.

57. The method according to claim 56, wherein the nucleic acid library comprises at least 1 clone of sequence selected from SEQ ID Nos: 1 to 37.

58. A kit for the study or assessment of the toxic potential of a test compound, said kit comprising a nucleic acid library comprising at least one nucleic acid clone specific for a gene selected from the following genes: Aldolase A; S4 subunit of proteasome 26S; Alpha-tubulin; Glucosidase II; lamin B receptor homologue; EF1-alpha; Fra-1; tyrosine kinase AX1 receptor; spliceosomale Protein SAP62; TRAF-3; EF2; TEF-5; CDC25b; interleukine-1 receptor-associated kinase (« IRAK »); WAF-1; c-fos (exon 4); ckshs1; PL16; NFAR-2; phosphatidylinositol4-kinase, ERF, Eph type receptor tyrosine kinase (hEphB1b); BAF60b protein of the SWI/SNF complex; EB1; MSS1; retinoïc acid alpha receptor (RARa); translation initiation factor eiF4A; STE20 type kinase; protein HSP 90kda; Lipocortin II; protein TPT1 (« translationally controlled tumor proteon ») Hsc70; Cytokeratin 18; 2-oxoglutarate dehydrogenase; mitochondrial gene NADH6; mitochondrial gene NADH deshydrogenase 4; alpha subunit of mitochondrial ATP synthase.

59. The kit according to claim 58, wherein the nucleic acid library comprises at least one clone of sequence selected from SEQ ID Nos: 1 to 37.

60. The kit according to claim 58, wherein the library is deposited on a support.

61. A nucleic acid library comprising at least one clone of sequence selected from SEQ ID Nos.: 1 to 37.

62. The nucleic acid library according to claim 61, wherein the library is deposited on a support.

63. A process of production of genetic markers of toxicity, the process comprising (i) hybridizing a nucleic acid population derived from cells in a situation of apoptosis and a nucleic acid population derived from cells in a control situation, (ii) isolating, from the hybrids formed in (i), clones characteristic of the situation of apoptosis, and (iii) hybridizing the clones obtained in (ii) with a nucleic acid sample derived from cells in a situation of toxicity.

64. A process of preparation of a DNA chip that can be used to diagnose the potential toxicity of a test compound, comprising the application on a

solid support of one or more nucleic acid libraries according to claim 61 or obtained by the process of claim 63.

65. A method for the identification of SNPs or other mutations or polymorphisms that allow the assessment of the response of a subject to a given compound, the method comprising (i) the identification *in vitro* of nucleic acids characteristic of splicing events induced in a cell treated with said compound and (ii) the identification of SNPs or other mutations or polymorphisms in the gene or genes corresponding to nucleic acids identified in (i), said SNPs or other mutations or polymorphisms allowing the assessment of the response of a subject to said given compound.

66. A method for the evaluation of the sensitivity or of the response of a subject to a test compound, comprising the analysis, from a biological sample comprising DNA from said subject, of the presence in the DNA of said subject of polymorphisms, SNPs, or other genomic alterations present in genes whose splicing is modified in response to said compound.

REMARKS

The specification was amended in order to incoorporate the priority information into the text. Claims 1-39 were canceled and replaced with claims 40-66 in order to place them in the most appropriate form for the U.S. No new matter has been added by any of the above amendments.

If there are any charges or credits not covered, please apply them to Deposit Account No. 03-2095.

Respectfully submitted,

Date: March 6, 2002

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